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## 5. INTRODUCTION

Women comprise 12.3% of the U.S. military active duty personnel, or approximately 200,000 servicewomen (as of June 30, 1993) (1). This is a significant number even compared to the 1,518,752 active duty men in military service, yet nutritional requirements of women have been far less studied than for men.

### **Energy Requirements in Women**

Although energy requirements of male soldiers have been and continue to be assessed by our labs (USARIEM and PBRC) and others under several environmental and training conditions using the doubly labeled water (DLW) method, energy requirements of female military personnel have not been studied.

Several studies which have included a subset of female subjects, have examined nutrient intake, which may give some idea of energy requirements. A recent assessment of intake was made in 49 Army women by a visual estimation method during an 8-week cycle of the Army Basic Combat Training course (2). Reported intake was  $2592 \pm 500$  kcal/d, which was within the range of energy intakes of 2000 - 2800 kcal/d for female soldiers ages 17-50 years old as defined by the Military Recommended Dietary Allowances MRDA (3). However, the range of intakes ranged from a low of 1294 to a high of 4388 kcal/d. Some of this is certainly due to errors in estimating energy intake, while some is due to true variations in intake. Energy deficit based on body composition changes averaged 180 kcal/d suggesting energy expenditures as high as 2800 kcal/d (4). Consumption of several micronutrients were less than adequate. Vitamin B<sub>6</sub> (76%), Folic acid (65%), calcium (73%), zinc (73%) and iron (90%) were each consumed at levels lower than that of the MRDA. These inadequate intakes point to a potential problem women may encounter when consuming military field rations. The nutrient density of these rations was designed with the higher energy requirements of males. A female recruit consuming meal ready to eat (MRE)s at an expenditure level of 2400 kcal/d would need to consume 131% of energy requirements to meet her daily needs for calcium and as high as 166% of energy requirements to meet her daily needs of iron. It may be necessary to supplement the rations with specific micronutrients to be used by those with lower energy intake requirements or design specific rations for smaller women soldiers.

The objective of the current study is to define a range of energy requirements of servicewomen, defining the variation (with adjustments made for body size/composition) as it relates to jobs, military settings, and activity patterns. This is crucial information needed not only for determination of nutritional requirements for energy balance, but specific nutrient density standards for servicewomen. This will address the first and third specific nutrition topics of the IOM report. Total daily energy expenditure will be measured using the doubly labeled water (DLW) method. As part of the DLW method, total water turnover can be calculated from deuterium elimination and total body water. Corrections are made for atmospheric water exchange, metabolic water and isotopic fractionation. From these calculations we can estimate actual fluid consumption in the field (the second nutrition topic) and fluid requirements during

specific categories of jobs and tasks (third nutrition topic). Activity patterns from actigraphs will be analyzed for hours of sleep, description of job/work patterns by examining bursts of concerted activity versus steady activity. Activity patterns will also be assessed using a boot insert which measures locomotory activity and voluntary energy expenditure. Men will also be studied in many of these settings. Energy requirements for men have been better established and will serve to anchor the results obtained in women to previously established norms in men (or confirm the validity of significant deviations also observed in the female data). We hypothesize that in some settings, there may be smaller differences between genders (normalized for fat free mass (FFM)) than in Army basic training, if absolute rather than relative, or ability group standards are emphasized. Such a finding would help demonstrate and explain a wider possible variation in female energy requirements.

## 6. BODY

### METHODS

#### Participants

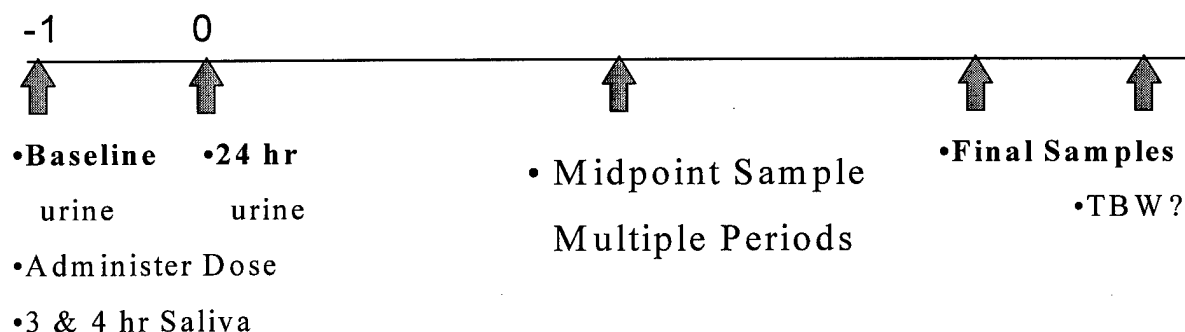
Subjects for these studies, with respect to gender, are as described above. We will attempt to recruit subjects to reflect the racial and ethnic makeup of the military units. This will generally include about 40% white and 40% black enrollment.

Inclusion Criteria would dictate that the volunteers be: within an age range is 18-35 inclusive, within 2 standard deviations for height and weight, female subjects must be non-pregnant and non-lactating, must be healthy with no pre-existing medical conditions, must be on no regular medication, must be able to communicate meaningfully with the investigator, be legally competent to give written and informed consent.

Exclusion Criteria would include volunteers with: Medical history of cancer, heart, kidney, liver disease or HIV or any other chronic or acute condition, thyroid dysfunction or any other significant endocrine abnormality, weight loss or gain greater than 10 pounds within the preceding two months.

#### DLW

A typical DLW protocol is depicted in the Figure below. The study begins with the collection of baseline urine and/or saliva samples, followed by oral administration of the  $^2\text{H}_2^{18}\text{O}$  dose. Saliva samples are obtained 2-4 hours after the dose for calculation of dilution spaces (5). A urine sample is collected the following morning for measurement of initial enrichment. Urine samples are then collected at the end of the period for measurement of final isotopic enrichment. The length of time a DLW study can be carried out depends on the turnover of the two isotopes, which is dependent on water and  $\text{CO}_2$  output. For studies in typical adults, the optimal metabolic period is 4-21 days (6), with Military Nutrition studies generally limited to the shorter time periods. If total body water (TBW) is expected to change over the metabolic period, subjects will need to be redosed with deuterium oxide at the end of the study for a final TBW measurement.



Since the DLW dose and the analyses are so expensive it is imperative that one obtains enough specimens to have backup samples at the beginning and end of the period of interest. Problems with specimens can occur in the field or in the lab, such as inability to obtain a specimen, loss of a specimen or contamination of a specimen. The protocol depicted in Figure 2 above illustrates the minimum number of specimens to be collected for a DLW study. This protocol provides backup samples for the initial and final time points. If there is a problem with the 24-hr urine, the 4 hr saliva specimen can be used as the initial time point. If the final sample is bad the urine from the previous day can be used as the final enrichment for elimination rate calculations.

The  $^{18}\text{O}$  isotope abundances will be measured on a Finnigan MAT 252 gas-inlet Isotope Ratio Mass Spectrometer with a  $\text{CO}_2$ -Water equilibration device (7). Briefly, urine and saliva samples will be equilibrated with  $\text{CO}_2$  at 18 degrees C in a shaking water bath for at least 8 h. The  $\text{CO}_2$  is then cryogenically purified under vacuum before introduction into the mass spectrometer. The hydrogen isotope abundances will be measured on a Finnigan MAT 252 gas-inlet Isotope Ratio mass Spectrometer, as previously described (7). Briefly, urine and saliva samples will be distilled under vacuum into Vycor tubes containing zinc reagent (Friends of Biogeochemistry, Bloomington, Indiana). The reduction tube will be sealed with a flame and placed in a 500 °C oven for 30 minutes to reduce the water to hydrogen gas which will then be introduced into the mass spectrometer. The  $^2\text{H}$  and  $^{18}\text{O}$  isotope elimination rates ( $k_{\text{H}}$  and  $k_{\text{O}}$ ) will be calculated using the isotopic enrichment relative to predose of the first day and each of the last two days of the metabolic study. During field studies, corrections for baseline isotope shifts from changes in water supply will be made by following a group not receiving isotope. Energy expenditure is calculated by multiplying  $\text{rCO}_2$  by the energy equivalent of  $\text{CO}_2$  for an assumed respiratory quotient (RQ) of 0.83 or that calculated from the food quotient (FQ) of the rations consumed and estimated changes in body energy stores during the study, as previously described (7).

The  $^2\text{H}$  and  $^{18}\text{O}$  isotope elimination rates ( $k_{\text{H}}$  and  $k_{\text{O}}$ ) can be calculated by the two-point method using the initial (i) isotopic enrichment and the final (f) enrichments:  $k = (\ln \text{atom percent excess (APE)}_f - \ln \text{APE}_i) / \Delta t$ .  $\text{CO}_2$  production will be calculated according to Schoeller et al. (6) as recently modified (8):

$$\text{rCO}_2 = (N/2.078)(1.007k_{\text{O}} - 1.041k_{\text{H}}) - 0.0246\text{rH}_2\text{O}_f$$

where N is the total body water calculated from the  $^{18}\text{O}$  enrichment in the 4 hour saliva (or average of initial and final 4 hr saliva samples if TBW is expected to change), and  $\text{rH}_2\text{O}_f$  is the rate of fractionated evaporative water loss which is estimated to be  $1.05N(1.007k_{\text{O}} - 1.041k_{\text{H}})$ .

## **Body Composition**

Height will be measured with a calibrated stadiometer on subjects in stocking feet and with heads positioned in the Frankfort plane. Body weights will be obtained from subjects in gym shorts and T-shirt using a calibrated electronic balance.

Body circumferences will be measured in accordance with the gender-specific methods of the Army, Navy, and Marine Corps, in every subject. (The Air Force is currently using the Navy equations.) Body fat calculations will be made for each of the service equations.

Body composition will be measured from measurement of body water from the doubly-labeled water technique to estimate fat-free mass in the subjects, using the fixed value of 0.73 for fat-free mass hydration.

## **Foot Strike Monitor**

Laboratory validation study design. Paralleling our earlier validation study (9), twenty military-eligible female volunteers will randomly divided into two equal groups: an equation derivation group, and an equation validation group. The two groups will be similar in age, height, and weight. The derivation group will be used to generate an equation describing the relationship between measured loco, assessed by indirect calorimetry, and the ratio of body weight to time of contact of the foot on the ground ( $Wb/tc$ ). This equation will be used to estimate loco from  $Wb/tc$  in a second group of subjects (the validation group). The validity of the equation will be tested by comparing measured loco and estimated loco.

Field training exercise (FTX) study design. Investigate the loco in subsets of women soldiers as a function of their natural military work environment. The body composition of each volunteer will be determined by anthropometry and isotope dilution immediately before and after about an eleven day FTX.

## **Activity Monitors**

Motionlogger Actigraphs, model AMA-32 (Precision Control Devices, Ft. Walton Beach, Fl.) will be employed to assess patterns of rest and activity, total physical activity and to estimate duration and fragmentation of sleep. This will permit comparison of these parameters across the various groups tested. These monitors have been used in previous military energy expenditure field studies to provide information on individual and group physical activity levels and patterns of activity and sleep in volunteers (10). Of particular interest will be gender differences in total physical activity and daily patterns of rest and activity. Such differences have previously been observed in healthy young men and women engaged in similar activities (11). The devices are 4 cm L x 3.1 cm W x 1 cm H, weigh 57 g and are worn on the wrist of the non-preferred hand using a standard wristwatch band. Each device contains a microcomputer, 32 k of memory, an analog-to-digital (A/D) converter and a piezoelectric sensor. They are powered by standard wristwatch batteries and can record continuously for over a week. Due to their small size and similarity to a wristwatch subjects do not find wearing them objectionable in any way. To obtain this high level of fidelity the monitors sample total activity counts in one-minute blocks of time.



Information on sleep patterns in the groups we propose to test is not currently available. Data collected by the AMA-32 will be downloaded to a laptop computer for further analysis using the ACTION 3 computer program (Ambulatory Monitoring, Inc.; Ardsley, NY).

## **TECHNICAL OBJECTIVES**

### **KEY OBJECTIVES**

- I. Define energy expenditure in servicewomen in various military settings.
- II. Determine if differences in total daily energy expenditure (TDEE) are explained primarily by differences in body size and fat-free mass after differences in activity patterns (locomotory and by wrist-worn actigraphy) are accounted for.
- III. Determine if the same holds true for differences between typical men, small men, and women.
- IV. Test methods which may be useful in prediction of TDEE.
- V. Assess hydration status of men and women by deuterium turnover (part of DLW).
- VI. Compare TDEE assessed by footstrike monitor to DLW.
  - A. Laboratory study: Demonstrate that the foot contact monitor (FCM) method provides valid estimates of the loco in military-eligible women over a full range of walking and running speeds, regardless of the phase of the menstrual cycle.
  - B. Field study: Establish the validity of estimates of total daily energy expenditure (estimated TDEE), calculated from FCM determinations of loco and resting metabolic rate, in female soldiers engaged in military training at the Marine Corps Mountain Warfare Training Center (MCMWTC), Bridgeport, California. The doubly labeled water measurements of TDEE will serve as a reference standard (measured TDEE).

We hypothesize that estimates of total daily energy expenditure of women soldiers in the field (estimated TDEE) will provide valid estimates of actual TDEE (measured TDEE). Valid estimates of TDEE by the Foot Contact Monitor/Resting Metabolic Rate method would suggest that minute-to-minute loco data can be used to estimate macronutrient requirements associated with military training in mountainous terrain. This type of information is urgently needed to improve the match between macronutrient demand and macronutrient availability from rations and body energy stores.

## **STATEMENT OF WORK**

Technical Objective: Determination Of Total Daily Energy Requirements, Water Turnover, and Activity Patterns of Servicewomen in Various Military Settings and Jobs

- I. Months 1-2: Preparation Phase
  - A. Protocol Development
  - B. Contact and clearly define FTXs

- C. Hire/Train Personnel
  - D. Order DLW dose for first year
  - E. Order Actigraphs and components for Foot Contact Monitor
  - F. Principal Investigators Meet to discuss and refine protocols
- II. Months 6-18: Army Basic Training Field Study
- A. Coordination Trip
  - B. Recruitment Trip
  - C. DLW dose preparation and shipment
  - D. Study team arrive and set up for field study
  - E. Conduct Energy Expenditure and Activity Pattern Study
  - F. Study team ship back equipment and samples
  - G. Isotope Analyses
  - H. Report Preparation
- III. Months 11-23: Marine Basic Training Field Study
- A. Coordination Trip
  - B. Recruitment Trip
  - C. DLW dose preparation and shipment
  - D. Study team arrive and set up for field study
  - E. Conduct Energy Expenditure and Activity Pattern Study
  - F. Study team ship back equipment and samples
  - G. Isotope Analyses
  - H. Report Preparation
- IV. Months 16-28: Mountain Warfare Training Field Study
- A. Coordination Trip
  - B. Recruitment Trip
  - C. DLW dose preparation and shipment
  - D. Study team arrive and set up for field study
  - E. Conduct Energy Expenditure and Activity Pattern Study
  - F. Study team ship back equipment and samples
  - G. Isotope Analyses
  - H. Report Preparation
- V. Months 20-32: Shipboard Field Study
- A. Coordination Trip
  - B. Recruitment Trip
  - C. DLW dose preparation and shipment
  - D. Study team arrive and set up for field study
  - E. Conduct Energy Expenditure and Activity Pattern Study
  - F. Study team ship back equipment and samples
  - G. Isotope Analyses
  - H. Report Preparation

- VI. Months 25-36: Army Units Field Study
  - A. Coordination Trip
  - B. Recruitment Trip
  - C. DLW dose preparation and shipment
  - D. Study team arrive and set up for field study
  - E. Conduct Energy Expenditure and Activity Pattern Study
  - F. Study team ship back equipment and samples
  - G. Isotope Analyses
  - H. Report Preparation
- VII. Months 34-36  
Prepare Final Report

### **SUMMARY OF PROGRESS**

- I. Months 1-2: First field training study identified, protocol developed, Personnel hired and trained, DLW dose water ordered, actigraphs ordered. We delayed purchasing new foot contact monitors as a new, improved version was being developed that is attached to the boot externally, so that we no longer have to have a custom boot insert made for the monitor. Therefore, for the first field training study, we used some of the old version that Reed Hoyt had on hand. We also delayed the validation study of the FCMs until the new version was received.
- II. Months 6-18: The first field study was conducted at Fort Bragg/Camp Mckall, NC, in a Combat Support Hospital field study. Isotope analyses are nearly complete, actigraph and FCM data are being analyzed.
- III. Months 11-23: We are in the process of identifying a population for the second field study. We have several leads but nothing definite at this point. The new FCMs will arrive and the laboratory validation study will be completed.

### **FIRST FIELD TRAINING STUDY**

This study was a combined effort of the Military Nutrition and Biochemistry Division, the Sustainability Directorate and the Science and Technology Directorate of the Natick Research, Development, & Engineering Center (NRDEC), and the Pennington Biomedical Research Center to assess the nutritional adequacy for women of the Meal, Ready-to-Eat ration during a field training exercise. The study will occurred during the field training exercise of a combat service support unit and investigated gender differences in food selection, nutrient intake, and energy expenditure.

### **TEST VOLUNTEERS**

Volunteers were recruited from the 28 Combat Support Hospital (CSH), Fort Bragg, that were engaging in a field training exercise of approximately 14-days duration starting on 1 May

1997. The CSH anticipated deploying almost half of its 520 personnel. This unit strength included 150 women, but did not include approximately 50 FORSCOM nurses that train with the unit. All soldiers from the unit who agreed to participate, except women who were pregnant, were included in the study.

Prior to the start of the study, the subjects were briefed on the nature and purpose of the study and the requirements for participation in the study and were familiarized with the experimental procedures. Subjects were informed verbally and in writing of their rights to withdraw from any part of the study without penalty or prejudice. The Commanding Officer of the prospective volunteers was informed of their responsibilities under AR 70-25 to ensure that the consent of any person under their authority to participate in this research is voluntary. Each subject completed a Volunteer Agreement Affidavit and Volunteer Subject Registry Data Sheet.

All volunteers were asked to participate in all data collection efforts. The volunteers were asked to complete questionnaires providing demographic information, medical history, diet history, nutrition knowledge and attitudes, to record all foods and fluids consumed for a total of seven days, and to record MRE lunches for an additional seven days. Individuals were asked to provide one blood sample and have body height taken once and body weights measured three times. A subsample of 32 volunteers were asked to participate in energy expenditure measures by a stable isotope technique and to wear wristband activity monitors and shoe liner foot contact monitors.

## STUDY CONDITIONS

The experimental test period were occur during a routine field training exercise in a temperate environment. The soldiers were provided three MREs per day for seven consecutive days during the field exercise. They were requested to eat no food other than that provided by the study team; however, the investigators were not take any enforcement measures. The importance of this restriction were explained to the CSH personnel at the orientation briefing. Bulk beverages or hot water typically available to combat service support personnel in the field were allowed.

A qualified medical monitor was supplied by the unit and was available during the entire experimental period. The medical monitor was responsible for terminating a volunteer's participation if medically indicated. Appropriate emergency medical service was available at Fort Bragg at all times during all tests.

## STUDY DESIGN

The data collection schedule is shown below. An orientation briefing was provided at the beginning of the study. Baseline assessments were conducted at this time. Baseline/descriptive measurements include: height, weight, body composition by skinfold measures, and blood chemistries. Demographics and nutrition knowledge questionnaires and the Diet Habit Survey were administered on the day of baseline measurements.

This collaborative study of women soldiers provided a unique opportunity to study their physiologic responses a multi-stress military training environment. The broad objectives were to: (1) quantitatively determine energy expenditure, and (2) use ambulatory monitoring technologies to make minute-to-minute measurements of soldier activity patterns and the metabolic cost of locomotion.

#### A. Test volunteers

20 women and 10 men dosed  
1 woman and 1 man undosed

30 volunteers, 2/3 female and 1/3 males, received doubly labeled water (DLW). The remaining 2 volunteers served as placebo controls. These subjects collected urine samples (saliva samples not necessary) at the same time as those drinking the DLW dose. This allowed for a correction factor to be calculated for any changes in isotopic baseline that might occur. Subjects were selected to obtain a variety of job classifications (MOS).

#### B. Experimental design

This study had a repeated measures design in which each test volunteer serves as his own control. The experimental design is outlined in Fig. 1 below.

Figure 1. Schedule of measurements.

	Days																
	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
MRE (+/- A-rations)			-	-	-	-	-	-	-	+	+	+	+	+	+	+	
Field training exercise	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
DLW/ <sup>2</sup> H <sub>2</sub> <sup>18</sup> O dose	x																
Saliva samples	x																
Urine samples	x	x	x						x	x	x		x		x	x	x
Food intake				x	x	x	x	x	x	x	x	x	x	x	x	x	
Body composition	x																x
Portable monitors*		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	

Note: DLW/<sup>2</sup>H<sub>2</sub><sup>18</sup>O dose = doubly labeled water, stable isotope labeled hydrogen and oxygen.

\*Portable monitors record activity and metabolic cost of locomotion.

#### PROGRESS

## 1. Doubly Labeled Water

All urine and saliva samples for the 30 dosed subjects and the 2 placebo subjects have been cleaned and prepared for isotope analyses. Deuterium and  $^{18}\text{O}$  analyses are nearly complete. Calculations and plots are being made to determine which, if any, samples need to be rerun. Final calculations of total body water (for EE calculations and for estimation of fat free mass), water turnover and total daily energy expenditures will be calculated in the next few months.

## 2. Activity monitor data

The actigraph and FCM data are in the process of being analyzed.

## 7. CONCLUSIONS

Overall this field study went very smoothly. We hope that the rest of the studies go as well. The final data analyses and report for this study will be completed in the time allotted in our Statement of Work.

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